Short Communication

Physiological variables and mitochondrial-related genotypes of an athlete who excels in both short and long-distance running

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Abstract

We report the athletic, physiological and mitochondrial-related genomic data of an Israeli endurance runner. He is holding the Israeli record in 10000, 5000, 1500 and 800m run, along with being one of the best Israeli 400m runners. We tested the *ACTN3* R577X, and six polymorphisms in the *PPARGC1A-NRF-TFAM* pathway genes. The case athlete was heterozygous for the *ACTN3* R577X variation and had five out of six 'endurance-oriented' genotypes, scoring significantly high in endurance 'optimal' genotype profile. In conclusion, we suggest that the case athlete is favoured by polygenic profile that is more suitable for mitochondrial biogenesis, regardless of his good phenotypic accomplishments in short-term running events.

1. Introduction

Elite athletic performance is a complex phenotype influenced by both environmental and genetic factors. Up to date the bulk of research indicates that the effect of a single gene variant on elite athletic status is rather small (Rankinen et al. 2010), and attaining elite status probably involves multiple genetic factors. To date, over 20 single nucleotide polymorphisms (SNPs) have been found to be individually associated with elite athletic status, ranging from endurance to sprint/power-oriented events (Ostrander et al. 2009). Among them are muscle-specific SNPs namely; the R577X substitution in the α-actinin-3 (*ACTN3*) gene (Druzhevskaya et al. 2008; Eynon et al. 2009e; Niemi and Majamaa, 2005; Yang et al. 2003), and the A/G SNP in the muscle-specific creatine kinase (CK-MM Ncol) gene (Santiago et al. 2010). However, it is still unknown whether different genetic variations and combinations play a role in specific athletic area (i.e. endurance performance or power-oriented performance) (Ruiz et al. 2009).

Metabolic phenotypes are essentially different between endurance and sprint/power athletic performance. Elite endurance performance requires enhanced oxidative capacity achieved by increasing several components of the mitochondrial respiratory chain (Mole et al. 1971) thus, resulting in high-levels of maximal oxygen consumption (VO_2max). VO_2max is arguably limited by cardiac output at least as much as it is by the capacity of skeletal muscle for oxidative phosphorylation. The muscle phenotype of endurance athletes is mainly composed of type I muscle fibers with a high mitochondrial density and size, which enables them to rely mainly on mitochondrial oxidation of carbohydrates and lipids for energy production . In contrast, short distance sprint and power performances are known to depend on anaerobic pathways which are especially dependent on intramuscular stored creatine

phosphate (CP), adenosine triphosphate (ATP), and glucose (Spencer and Gastin 2001). Consequently, it is unusual to identify athletes who excel in short sprints as well as in long distance running. This theory was well supported by Van Damme and colleagues, who demonstrated that in world-class decathletes results in explosive power events such as the 100-m sprint or the long jump, are negatively correlated with endurance-oriented event such as the 1,500-m race (Van Damme et al. 2002). Given the aforementioned phenotype differences between endurance and sprint/power athletes, it is presumed that the 'optimum' genotype profile also differs between these two types of athletes.

Evidence from both human (Mahoney et al. 2005; Norrbom et al. 2004; Russell et al. 2003; Short et al. 2003) and animal studies (Baar et al. 2002; Terada et al. 2002) imply that transcription factors and co-activators in the peroxisome proliferator-activated receptor (PPAR)-nuclear respiratory factor (NRF)-mitochondrial transcription Factor A (TFAM) pathway influence endurance performance, due to their key role in regulation of cellular energy metabolism. Previous studies from our laboratory indicated that the R577X substitution in the *ACTN3* gene (Eynon et al. 2009e) and several variants in the *PPARGC1A-NRF-TFAM* pathway (Eynon et al. 2009a; 2009b; 2009c; 2009d;) are associated with elite athletic status. We also suggested that in general, endurance athletes have a polygenic profile that is more adjusted for mitochondrial biogenesis, as opposed to power athletes or non-athletic controls (Eynon et al. 2010).

Here we report the specific physiological variables and the mitochondrial-related genomic predisposition of a track athlete who successfully competes in both short and long running distances that are considered to represent two physiological end-points of the human performance continuum.

2. Case Report

We studied an Israeli endurance runner (age=36 years, height=180 cm, weight=65 kg, body fat percentage=8%) whose main event is the 10000m race. He is holding the Israeli record in 10,000, 5000, 3000, 1500 and 800m running events, but also routinely run the 400m race at national and international track and field meets. His personal best running times are reported in Table 1. We genotyped six genetic SNPs in the *PPARGC1A-NRF-TFAM* pathway genes, which are known to be associated with endurance exercise performance capacity: (i) *NRF2* A/C (rs12594956) (Eynon et al. 2009a); (ii) *NRF2* A/G (rs7181866) (Eynon et al. 2009d; He et al. 2008); (iii) *NRF2* C/T (rs8031031) (Eynon et al. 2009a); (iv) peroxisome proliferator-activated receptor alpha (*PPARA)* intron 7 G/C (rs4253778) (Ahmetov et al. 2006; Eynon et al. 2009b); (v) peroxisome proliferator-activated delta (*PPARD*) C294T (Eynon et al. 2009c); (Skogsberg et al. 2003); and (vi) *PPARGC1A* Gly482Ser (Eynon et al. 2009c; Lucia et al. 2005). We also studied the *ACTN3* R577X polymorphism, which is known to be associated with power-oriented performance (Druzhevskaya et al. 2008; Eynon et al. 2009e; Niemi and Majamaa, 2005; Yang et al. 2003). The studied polymorphisms and genotypes of the Israeli endurance runner are presented in Table 2.

2.1 Graded exercise tests

The subject came to the laboratory twice in order to perform two different tests. During the first session an incremental maximal running test was carried out on a motor-driven treadmill (Woodway, Germany), in order to determine his maximal oxygen uptake (VO_2 max). The initial speed was 13 km/h and the grade was 1%. Speed was increased by 1 km/h every minute while grade was maintained at 1% until the fifth stage (5th min). From this point on, inclination was increased by 2% every

minute while speed was kept constant until volitional exhaustion. Expired gases and respiratory exchange ratio (RER) were measured breath by breath throughout the test, using an automated on-line metabolic analysis system (SensorMedics Vmax 29, USA).

In the second session, the lactate threshold (LT) was determined by monitoring blood lactate concentration during a graded running test. Initial running velocity was 14 km/h and increased by 1 km/h every 4 minutes. A capillary blood sample was collected from a preheated fingertip at the end of each stage and analyzed using a portable lactate analyzer (Accusport, Boehringer Mannehein, Germany). Lactate concentration values were plotted against running velocity and the LT was determined at the point at which inflexion of the lactate curve occurred.

During both tests heart rate was recorded at each stage using a Polar heart rate monitor (Polar Accurex Plus, Polar Electro, Woodbury, NY). Rate of perceived exertion (RPE) was determined using the original 6-20 Borg scale at the end of each stage.

2.2 Genotyping

Genomic DNA was extracted from peripheral EDTA treated anti-coagulated blood using a standard protocol. Genotype analyses were performed using the polymerase chain reaction (PCR) following restriction fragment length polymorphism (RFLP) analysis as previously described (Eynon et al. 2009a; 2009b; 2009c; 2009d; 2009e). To ensure proper internal control, for each genotype analysis we used known positive and negative controls from previously genotyped samples. The results were scored by two experienced and independent investigators who were blind to subject data.

The study was approved by the Helsinki Committee, the formal ethics committee of the Hillel Yaffe Medical Center, Hadera, Israel, according to the Helsinki Declaration.

Written informed consent was obtained from the participant. The athlete consented to being identified in this way, and thus publicizing his genetic and other data.

3. Results

3.1 Physiological variables

The athlete completed the exercise test without any complication. He exercised to exhaustion and stopped when he reached maximal effort and fatigue as evidenced by perceived exertion (RPE 19-20). His VO_2max (which corresponded to a speed of 20.3 km/h) was 80.4 $ml \cdot kg^{-1} \cdot min^{-1}$, while criteria of maximal effort attainment were met (maximal value of HR of 187 beats/min, blood lactate concentration at end-exercise of 11 mmol/L, and respiratory exchange ratio > 1.1) (Lucia et al. 2006). The running speed eliciting the LT was 19 km/h.

3.2 *ACTN3* and mitochondrial biogenesis-related genotypes

The case athlete had five out of six "optimal" endurance (related to mitochondrial biogenesis) genotypes (Table 2), (Eynon et al.2010) and was heterozygous for the *ACTN3* R577X genotype (i.e.; harboured the RX genotype). It is also noteworthy that none of the other endurance athletes we previously studied had an ‘optimal’ (six out of six) endurance genotype profile, i.e. theoretically most favourable for mitochondrial biogenesis (Eynon et al. 2010). Taken together the present data and those we recently published (Eynon et al. 2010), it seems that the probability of occurrence of the theoretically optimum genotype profile is very rare, at least in terms of mitochondrial-related genes. The comparison between the endurance "optimal" genotype and the "power-oriented" genotypes is presented in Table 2.

4. Discussion

We examined the endurance polygenic profile of six polymorphisms in the *PPAR-NRF-TFAM* genes pathway, and the R577X variation in the *ACTN3* gene, as well as physiological variables of an endurance runner who excel in short as well as in long distance running. Our main finding was that the subject has a theoretically mitochondrial-related genotype that is more suitable for endurance-type events. This finding is unexpected due to his ability to outperform also in a short and mid-term running events, i.e. 400m and 800m , which probably are different from longer events both in physiological and genotype profiles.

The most extensively studied genetic polymorphism by far; in the context of power-oriented athletic performance is the *ACTN3* R577X. It has been demonstrated that the RR genotype is over-represented in several groups of power-athletes (Druzhevskaya et al. 2008; Eynon et al. 2009e; Niemi and Majamaa, 2005; Yang et al. 2003), with the overall conclusion that this specific genotype may confer an advantage in power-oriented sports events, e.g. sprint, jumping or throwing. This observation, along with the notion that the case-athlete was heterozygous for this polymorphism , leads to believe that, at least in terms of this specific SNP, the athlete does not have the most suitable genotype for becoming an elite-level sprinter.

Although the athletes' VO_2max approached the highest reported values for humans, representing a high potential for endurance performance, he held also the national record in 800m, and was also positioned among the best 400m runners in Israel. This phenomenon is very uncommon among runners, but not so uncommon in swimming. This can be seen from the records of top world class-swimmers, such as Ian Thorpe (world record holder in 100-m relay, and individual 200, 400, and 800-m swims), and Grant Hackett (world record holder in 200-m relay, and individual 400,

800, and 1500-m swims), as well as others who excel in both short and long swimming distances. This may be at least partially explained by the usual emphasis put on high volume training loads by swimming coaches , regardless of the swimmer's event specialty.

Elite athletes represent a somewhat unique model of study. These individuals have undergone extreme physiological adaptations (e.g. in muscle oxidative capacity, as a result of increased mitochondrial content) that are likely to be the consequence of years of training as well as of the interaction between exercise training and a favorable polygenic profile (Ruiz et al. 2009). In the present study, our case athlete represents even more "exceptional" model by holding the all times Israeli national records in 10,000m and 800 m running events, and by being one of the best Israeli 400m runners.

A continuous near-maximal (>90% VO_2max), and a supra-maximal efforts are required in 10,000 m and 400m track events respectively (Rubio et al. 2005). In the performance of both types of running events a highly adapted glycolysis metabolic pathway at the muscle level might explain the aforementioned phenomenon.

One limitation of this report lies on the fact that the case-athlete belongs to a very unique minority group of Ethiopian Jews. Between the years 1977-1991, 27000 Ethiopian Jews immigrated to Israel. During the last 34 years their only influence on the Israeli track & field record table was in the half marathon and marathon run, and none belonged to the top ten all times ranking in 800m, 1500m and 3000m running events. Moreover, in recent years, we collected DNA samples from eight long-distance Ethiopian Jews runners and found that their genotype profile was similar to that of native Israeli long-distance runners (unpublished data).

We are also aware that compared to the world record list, the athlete's results are not so impressing. However, the case-athlete is very unique in his own country, and we are not aware of any other country in the world in which the national record holder in 800m through 10000m run is the same person, and the same athlete is also ranked at the top four of the nation's all times results in the half marathon run.

In conclusion, we suggest that the case athlete is, in general, favoured by a physiological profile and polygenic endowment that are more suitable for mitochondrial biogenesis, which could apply also for his good result in 400m and 800m run. This case study represents a model of how evaluation of the polygenic profile can assist athletes and their coaches in the preparation of a training program and in track specialty selection. It is well known that aerobic and anaerobic specificity develops toward the end of puberty (Bar-or & Rowland, 2004), and that during early puberty athletes may excel in both short and long distance events. Thus, early information regarding the polygenetic profile can direct the athlete and his/her coach towards the sports event for which he is best suited (e.g. aerobic versus anaerobic). However, it is still premature and speculative to predict the possibility of one becoming an endurance or sprint/power athletic champion based on genetic data.

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Tables:

Table 1. Athletes' personal best running times.

Table 2. Studied polymorphisms and genotype of the case study athlete (Israeli endurance athlete).